ANATOMICAL RESPONSES OF A GRASSLAND VEGETATION TO AIR POLLUTION

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by

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IN THE NAME OF ALLAH,

THE BENEFICIENT,

THE MERCIFUL.

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CERTIFICATE

This is to certify that the dissertation entitled 'ANATOMICAL RESPONSES OF A GRASSLAND VEGETATION TO AIR POLLUTION' is a bonafide work carried out under my supervision by Miss Naheed Rabab Usmani and can be submitted in partial fulfilment of the requirements for the award of the degree of Master of Philosophy in Botany.

(A.K.M. GHOUSE)
Professor

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INTRODUCTION

One of the most important and vexing challenges in the field of public health is 'Environmental Pollution'. Environmental pollution may be defined as the direct or indirect change in the ecosystem which is harmful to the system of living beings (i.e. animals and plants), and produces undesirable effects. In the words of Sir Fedrick Warner, 'a substance is normally considered to be a pollutant, if it adversely alters the environment by changing the growth rate of species, interferes with the food chain, is toxic or interferes with the health, comfort, amenities or property values of people'. In view of the aforesaid statements, 'maintainence of a clean environment and ecological balance' are subjects of vital and world-wide importance.

Eversince man has become aware of the deleterious effects inflicted by him to his habitat due to his indiscriminate interference witn nature he has tried to work for the betterment of his environment. Following the clean Air Act of 1956, (in which burning of coal was banned), scientists, ecologists, engineers and anthropologists have taken up tasks of reducing the hazards caused by air, water and soil pollutants. Berry et al., (1974) in their studies, dealt with every aspect of pollution and showed a correlation between the level of pollution and its effect on human health. The pollutants enter the human body, resulting in the development of blood and lymph node cancer, tissue changes in brain, liver and kidneys, asthema and hundreds of other such ailments. In India, several programmes regarding control of pollution have been taken up by the 'National Environmental Engineering Research Institute', Nagpur; Department of Environment, New Delhi, and similar other institutes.

Approximately 175 million hectares of land, (i.e. 53% of India's total land area) is under the influence of serious environmental degradation. To cite an example of the devastating effect of soil pollution, Cherapungee, which was once the wettest region in India, is now a barren land (Khator, 1984). Studies have shown that air pollution is affecting plant life in the same manner as it is affecting animal life. The vegetation is susceptible to injury, which may be localized, with the reaction restricted to the exposed area, or the whole plant may be adversely affected. In many cases, plant damage is a far more sensitive and reliable indication of the degree of air pollution, than any other test performed. Liverworts, such as Anthoceros respond to slightest increase in sulphur-dioxide content in the air.

Pollution affects plants in two ways:

(i) By disturbing the physiological activity of the plant thereby hampering growth, without causing any visible damage to the plant.

(ii) By causing visible damage to the plant such as necrosis on the foliar parts, decrease in fruit production etc.

Studies done by Rao, 1971; Leblance and Rao, 1975; Tourangeau et al., 1977; Zaidi et al., 1979; Ghouse et al., 1980; Ashenden and Williams, 1980; Barton et al., 1980; Borka, 1980; Ferguson and Lee, 1980; Lal and Ambasht, 1980, 1981; Ambasht, 1981; Hagver and Kjondal, 1981; Vollmer et al., 1982; Ghouse and Khan, 1983 a,b,c,d,e; Ghouse et al., 1984; have revealed the injurious effect of air pollution on plants.

Susceptibility of different plants to air pollution shows a varience. Table 1 shows the various plants according to their responses to pollutants.

TABLE - 1[1]

Sensitive plants	Intermediate plants	Resistant plants
Alfalfa	Castor bean	Corn
Barley	Arhar	Lily
Soyabean	Rose	Citrus
Wheat	Guava	Babul
Pumpkins	Jamun	Shisham
Onions	Neem	Tulsi
Mango	Eucalyptus	Ashok
Peas		Ber
Tomato		

1[1]. Kumra, V.K., Kanpur City: A Study in Environmental Pollution, 1982, pp. 114.

Coal smoke and gases have occupied the center of the stage almost exclusively in many industrialized areas of the world, and are still a dominant concern. A survey of literature indicates that pollutants are posing a threat to the very survival of vegetable crops, weeds and timber trees. (Gupta, 1981; Amani, 1982; Khan, 1982; Ghouse et al., 1983 a,b). The present study has been proposed to be undertaken on herbaceous flora of Kasimpur, which is under the influence of noxious gases emitted from the Thermal Power Station situated there.

The Thermal Power Complex is situated about 16 kms. North-East of Aligarh. There are three power plants, namely stations 'A', 'B' and 'C'. On an average, there is a consumption of 3,192 metric tons of butaminous type of coal, daily. Its combustion produces mainly SO_2, CO_2 and NO_2 along with other gases and particulate matters.

CONCEPT OF AIR POLLUTION

The term 'smog' originated in Great Britain and seems to have been in common use before World War I. The term was suggested by H.A. Des Voeux's 1911 report to the Manchester Conference of the Smoke Abatement League of Great Britain, concerning smoke-fog deaths that occurred in Glasgow, Scotland in 1909. Such deaths were estimated to be 1063 in number.

Inspite of the recent rapid shift towards harvesting solar energy, waste gases, coal dust and ash arising due to coal burning, still contribute to a large extent to the pollution of the atmosphere.

According to Seamans (1956) "Air pollution can be defined as the presence in the atmosphere, of substances, resulting from the acts of man, in quantities which are or may become injurious to human, plant, or animal life, or to human property".

As defined by Engineers Joint Council, air pollution means the presence in the out-door atmosphere of one or more contamination, such as dust, fumes, gases, mist, odour, smoke, and/or vapour in quantities and characteristics and duration as to be injurious to plant, human and animal life, and to property, or which unreasonably interferes with the comfortable enjoyment of life and property. (Bishop, 1957).

Some major pollution sources are as follows:-
(a) Vehicles, (b) Electric power generation, (c) Industrial and domestic fuel burning, (d) Refuse disposal and burning (e) Industrial processes.

Air trapped under the upper layers of the atmosphere prevents the vertical rise of pollutants, thereby causing local concentrations of such pollutants over cities like Bombay, Madras, Kanpur, Calcutta and Delhi. Table 2 shows the major air pollutants and their sources of emission.

TABLE - 2*

Pollutant	Major sources
Ammonia	Ammonia and urea plants.
Arsenic	Gas purification plant in ammonia and urea manufacturing.
Boron	Manufacturing of synthetic boron.
Carbon-monoxide	Transportation, fuel combustion, industrial processes and garbage disposal.
Carbon-dioxide	Transportation, fuel combustion, industrial processes.
Colour	Various process plants.
Dust	Various plants using coal and automobiles.
Fluorides	Superphosphate plants.
Lead	Various industrial processes and automobiles.
Nitrogen-dioxide	Transportation, fuel combustion, processing and solid waste disposal.
Phosphate	Phosphoric acid and complex fertilizer plants.
Sulphur-dioxide	Transportation, industrial processes, solid waste disposal.

Table - 2* - Pandey, G.N., "An Introduction to Environmental Control", paper read on Environmental Pollution Control, H.B.T.I., Kanpur, 1976, pp. 9.

CONCEPT OF INJURIES AND DAMAGE

Air pollutants cause two types of damage to vegetation: (a) internal damage to the plant affecting the overall life processes of the plant, (b) external damage to the plant, appearing as lesions, decrease in chlorophyll content, etc.

Regarding the type of reaction appearing on the plant due to the pollutants, two terms have been loosely used, and often considered to be synonymous. They are 'injury' and 'damage'.

Guderian et al., (1960) made a distinction between 'injury' and 'damage' caused by air pollution. According to them, 'injury' can be defined as any identifiable and measurable response of a plant to air pollution, while 'damage' is any identifiable and measurable adverse effect upon the produce of a plant which results from an air pollutant injury. This means that an injury caused to a plant due to air pollution may result in producing some damage such as reduced leaf area, stunted overall plant growth etc.

OCCURRENCE OF INJURY AND DAMAGE:

In spinach, the abaxial leaf surface assumes an oily appearance first, as injury due to air pollution manifests itself. This is followed by leaf bronzing and collapse of epidermal cells. This pattern of injury is also shown by Romaine lettuce, beets, celery, and many other plants. Thus injury and damage occur together (Brandt and Heck, 1968).

Gupta (1981), showed a relation between the percentage of leaf destruction and the total yield of some vegetable crops. Reduced growth rings in forest trees were noticed by Ghouse et al. (1983 a,b) and such reduction can be compared to leaf injury as observed by Brandt and Heck (1968).

AIR POLLUTION INJURY SYMPTOMS ON VEGETATION:

Though not mutually exclusive, the general visible injury symptoms on the foliar parts of plants can be catagorised under the following headings:-

(a) Leaf tissue collapse with necrotic patterns.
(b) Chlorosis or other colour changes.
(c) Growth alterations.

However, the above mentioned injury patterns cannot be solely attributed to the action of air pollution, since insects, disease and nutritional def-iciencies can also produce similar leaf patterns. Thus to properly diagnose air pollution effects on vegetation, the problem must, therefore, be seen in the field and supported by measurements of the concentration of suspected pollutants in the air; furthermore, a thorough knowledge of local cultural conditions is required.

(a) LEAF TISSUE COLLAPSE WITH NECROTIC PATTERNS:

Peroxy-acetyl nitrate (PAN), ozone, fluoride and sulphur-dioxide injury results in plasmolysis of cells and finally collapse of the tissue. The extent to which the neighbouring cells are damaged depends upon the concentration of the toxicant. The affected regions on the leaf first appear as slightly water-soaked and bruised areas, which later, become dry, forming necrotic patterns characteristic of the toxicant.

(b) CHLOROSIS AND OTHER COLOUR PATTERNS:

Chlorosis, (i.e. loss or reduction of chlorophyll) is a very common indication of air pollution injury. Chlorosis usually results in a pale green or yellow colour of the leaves. Its appearance indicates some nutritional def-iciency of the plant.

Chlorosis may appear in association with injured and collapsed tissues. Such an incidence may be seen in the case of fluoride burn, when a narrow margin of chlorosis surrounds the necrotic lesions. Blotchy, chlorosis patterns appear along with blotchy, collapsed areas in case of sulphur-dioxide injury. In corn, where flouride burn occurs, scattered chlorotic flecks occur at the tips and upper margins of the leaves. This typical positional location of chlorotic pattern distinguishes it from chlorosis occurring due to other factors. Sometimes, the oxidant type of smog also produces chlorosis.

Besides chlorosis, other colourations also make their appearance on the leaves. Sulphur-dioxide causes bleaching of the tissues, while a brown colouration occurs due to fluorides. Silvering or bronzing of the abaxial side of leaves is associated with PAN injury.

(C) GROWTH ALTERATIONS

Most air pollutants have the property of affecting the action of hormones, thereby interfering with the growth patterns of the plants. Abnormalities produced as a result of this are usually twisting and/or elongation of both leaves and stems.

Ethylene induces epinasty, or drooping of the leaves, as well as leaf abscission. Young cotton bolls show damaging abscission, and loss of apical dominance (Hall et al., 1957). A stunting of growth has been observed in plants in the United States and England. Smog components (ozone, PAN, nitrogen-dioxide) cause formation of smaller fruits, early senescence. Fluoride burn which causes leaf destruction, results in dieback of branches, since the functioning of the leaf is hampered. Some abnormalities produced by air pollution, such as early leaf drop, changes in water relations, increased respiration and stunted growth have been shown experimentally by Gupta (1981), Khan (1982). Bokra (1980) reported reduced respiration rate and enzyme catalase activity.

Ghouse and Khan (1983 a,b,c,d,e) worked on certain perennial and annual weeds and found that their growth in terms of green area, dry matter accumulation, fruit set, root and shoot length, and biomass was greatly impaired in polluted atmosphere.

Similar results were obtained by Khan and Khair in 1984 a,b,c, on certain weeds of a grassland vegetation. Ghouse and Amani (1978) found that air pollution had an adverse effect on fruit formation and seed setting in Dalbergia sissoo.

However, the effects of air pollution may not always be adverse. To cite an example, data obtained by Amani et al. (1978) on Cassia occidentalis and Cassia tora, a remarkable increase in the vegetative and reproductive growth of the plants have been demonstrated. The size and weight of the plant, shoot, root, fruit and leaves showed an increase over that of plants growing in ambient air. This observation can be attributed to the fact that these plants make use of the carbon-dioxide rich atmosphere of the polluted site.

SEVERITY OF INJURY

The extent to which injury is caused depends upon the concentration of the pollutants. In general, three categories can be made, regarding the degree of injury:

(i) Transient injury:- When the concentration of pollutants is relatively low, chloroplasts of the cells remain intact and the injured cell may recover and resume normalcy.

(ii) Chronic injury:- Some cells may get injured or killed without showing apparent collapse of tissue, but there is lightening of colour in this case.

(iii) Acute injury:- When the concentration of pollutants is high, the exposed cells as well as the neighbouring cells are killed.

Chronic injury may be caused due to absorption of pollutants over a long stretch of time (Thomas, 1951 ; Thomas and Hendricks 1958). Chronic injury results from prolonged exposure to very low concentrations of sulphur-dioxide in the order of 0.1 to 0.3 ppm.

HIDDEN INJURY:

Stoklasa (1923) put forward the concept of hidden injury for the first time. It means that there is less crop yield caused due to air pollution, without any visible injury symptoms shown by the plants. This is brought about by upsetting the basic life processes of the plant by the pollutants, thereby disturbing the overall physiology. Work done by Hill and Thomas (1933) shows that the degree of alfalfa yield reduction by sulphur-dioxide is directly proportional to the degree of leaf surface area destroyed. Thomas (1958), showed that sulphur-dioxide affected photosynthesis, though it is not apparent outwardly. Yield of tomato showed a loss due to hidden damage caused by flourides (Hill et al., 1958). Todd (1956) observed retarded leaf growth, premature senescence and loss in productivity, without any visible leaf symptoms on bean plants. Gittenden and Read (1978) too, found decrease in dry matter production in Lolium perenne. Carlson (1983) found that when sulphur-dioxide concentration exceeded 0.8 ppm, the mesophyll conductance to carbon-dioxide decreased.

Though the use of the phrase 'Hidden Injury' has been in vogue, it is not very accurate, because, had the injury been 'hidden' or 'invisible', a loss cannot be measured. So it is more appropriate to denote it as 'Physiological Injury'.

ACID RAIN:

Various oxides of carbon, sulphur and nitrogen, present in the atmosphere, dissolve in water to produce their respective acids. This acid, when brought down to the soil is known as acid rain. Natural rain has the pH of about 5.6, and is not neutral (i.e. its pH is not equal to 7.0). This is because of the presence of weak acids such as carbonic acid formed by the combination of carbon-dioxide of the atmosphere with water. Such a rainfall having the pH values less than 5.6 is known as acid rain. There can be acid snow, acid hail, acid sleet, and even acid fog.

Acid rain effects top soil and decreases the rate of decomposition of the forest floor litter, inhibits nitrogen fixation, enhances the weathering of rocks. As a consequence of this, the nutrient cycles in agricultural and forest lands are disturbed, and hence, there is lowering of fertility over long term (Hagner and Kjondal, 1981; Kumar and Sharma, 1981). These are the indirect, adverse effects of acid rain on plants.

Acid rain effects vegetation directly also. It can accelerate cuticular erosion, thereby facilitating easy penetration of tissues by pathogens and saprophytes. Acid rain can cause plasmolysis of palisade cells, chloroplast damage and consequently, photosynthetic process is also affected. In case there is an increase in rain acidity, the foliar leaching of nutrients is also increased.

REVIEW OF LITERATURE

Power generation contributed to about 14.2% of the total air pollutants (Hesketh, 1973) in 1968. Of this, sulphur-dioxide was the main pollutant, having a percentage of about 8.5, while the remaining 5.7% was contributed by oxides of nitrogen (2.2%), carbon mono-oxide (0.7%), hydrocarbons (0.7%) and particulate matters (2.1%).

Sulphur-dioxide being the prime pollutant, the literature concerning it has been reviewed in greater detail as compared to that of other,pollutants.

The major air pollutants can be listed as:-

(1) Sulphur dioxide
(2) Oxides of nitrogen
(3) Hydrogen fluoride
(4) Ozone
(5) Dust in the air

Minor pollutants:-

(1) Ethylene
(2) Ammonia
(3) Herbicides.

SULPHUR-DIOXIDE

Sulphur-dioxide occurs at a concentration of several ppm by volume in contaminated atmosphere of cities (Cholak, 1952).

The main source of sulphur-dioxide is the burning of fuel. A power station burning 5,000 tonnes of coal/day, discharges 500 tonnes of sulphur-dioxide into the air (Johnstone, 1952). 60% of sulphur-dioxide is produced by coal burning in thermal power stations alone, 20.7% is enamated from oil refineries, and the rest comes from various domestic combustion process (Rohrman and Ludwig, 1965), Sulphur-dioxide dissolves in water (Terraglio and Manganelli, 1967). Engdahl (1962) showed that the formation of sulphuric acid in droplets was greatly accelerated by the presence of certain metallic oxides such as those of manganese and iron. Thomas (1961) showed that conversion of sulphur-dioxide to sulphuric acid was very rapid when wind velocity was low and oxidants high.

MECHANISM OF INJURY BY SULPHUR-DIOXIDE:

The concentration of sulphur-dioxide to which the plant is exposed depends on the concentration at the source and second on the degree of dilution in transit. Once it falls on the vegetative canopy, sulphur-dioxide may be directly absorbed by the plants. Large amounts are absorbed by the aerial parts of the plant. Fried (1949) demonstrated that alfalfa plants and lemon trees absorb sulphur-dioxide directly through the leaves and utilized it; but most of the sulphur probably settles on the foliage and is ultimately washed to the ground. When plant tissues accumulate sulphur-dioxide faster that it can be oxidized and assimilated, phytotoxic concentrations arise in intercellular spaces on the leaf, causing cell injury (Thomas, 1961).

The toxic nature of sulphur-dioxide is attributed to its reducing property (Bleasdale, 1952). There is generally an equilibrium between sulphydryl groups and the more oxidized sulphur compound, e.g. sulphites. Any unbalance in this equilibrium, caused by excess of oxidized sulphur compounds, upsets sulphur utilization and protein synthesis. Sulphur-dioxide reduces the sulphites to sulfhydryl, causing an accumulation of sulfhydryl and an imbalance in the ratio of sulfhydryls to oxidized sulphur compounds. The tolerance of plants to sulphur-dioxide may be a function of the stability of sulfhydryl.

PLANT RESPONSES:

The basic cellular responses to sulphur-dioxide are the same for all species, but due to the differences in anatomical characters, the symptoms developed, differ markedly from each other.

SYMPTOMS SHOWN BY NEEDLE-LEAVED SPECIES:

Evergreen trees, having needle-leaves show pronounced discolouration of their leaves, which turn reddish-brown in colour. Shrinkage of tissues, followed by early defoliation is noted. Katz et al. (1939), found that Doughlas fir needles shed within a few days or weeks, as a result of an injurious exposure to sulphur-dioxide.

Necrosis of needle-leaves may appear as tip burn, banding or basal burn. Necrosis and chlorosis often develop on scattered needles in a fasicle often develop on scattered needles in a fasicle rather than on all the needles of a shoot.

Needle damage and defoliation are pronounced more on the current year's needles at the apices of the branches. Older leaves have lower metabolic activity and are thus not affected much. Katz (1939) ranked some conifers in order of their decreasing susceptibility as follows:- Western larch, Douglas fir, Yellow pine, Engelmann spruce, White pine, Hemlock, Lodgepole pine, Silver fir, White fir and Red cedar. Junipers are among the most tolerant species.

SYMPTOMS SHOWN BY BROAD-LEAVED SPECIES:

(DICOT PLANTS):

Alfalfa is one of the most sensitive plants to sulphur-dioxide action, and sulphur-dioxide concentrations of the order 0.3 to 0.5 ppm produce acute necrosis of intercostal type. Such lesions may have a chlorotic border.

In case necrosis is along the veins, it may be in the form of a 'Christmas tree' pattern. Prolonged exposures to low concentrations of sulphur-dioxide, in the order of 0.1 to 0.3 ppm produces chronic injury, which appears as blotchy chlorosis. When mild, chlorosis appears, leaves may return to normal in a day or two, and when it is severe, chlorophyll is completely destroyed and leaves turn yellow or brown.

Broad leaved trees and shrubs are generally more tolerant to sulphur-dioxide than conifers.

MONOCOT PLANTS:

Cereal crops, such as barley are highly sensitive to sulphur-dioxide and readily damaged by sulphur-dioxide concentration of 0.3 to 0.5 ppm. The leaf tips first turn grey-green. Chloroplasts break down and affected tissues become flaccid, and if exposed to sunlight, shrink rapidly and get bleached. Young leaves are most susceptible at the tips, while the old bent-over leaves are sensitive at the portions where they bend.

Tip and marginal necrosis is often accompanied by spotted, interveinal flecks or lesions between the midrib and margins.

EFFECT OF SULPHUR-DIOXIDE ON GROWTH AND PHYSIOLOGY OF PLANTS:

As a consequence of damage caused by sulphur-dioxide, the overall physiology and hence the growth of the plant is affected. When photosynthesis, respiration, permeability stomatal movement and water relations are slowed down, growth also decreases.

Hill and Thomas (1933), showed that yield loss in alfalfa plants, which were fumigated with a series of dozes of sulphur-dioxide, was directly proportional to leaf necrosis. Brisley and Jones (1950) found that loss in yield of a crop of wheat generally ranged from 0.26 to 0.62% for each 1% of foliar damage.

In order to see whether sulphur-dioxide restricts growth and physiology in absence of chlorosis and necrosis, Julius Stoklasa, in 1923, postulated that toxic gases at concentrations below those causing any visible symptoms, caused a reduction of photosynthesis, early senescence an upthrifty appearance, reduced growth and yield and increased susceptibility to disease and insects. This is termed as 'physiological injury'. Similar conclusions regarding impairment of growth and other metabolic processes by sulphur-dioxide were drawn by

Katz (1952), Grayson (1956) and Linzon (1958).

Koziol and Cowling (1981) reported a loss in the production of epicuticular wax in Lolium perenne due to sulphur-dioxide pollution. Garsed et al. (1981) found growth reductions in scots pine due to prolonged exposures of 143 $gm^{-3}SO_2$. Gilbert (1983) has reported that the annual ring width of Pinus nigra was considerably decreased due to pollution by sulphur-dioxide.

EFFECT OF SULPHUR-DIOXIDE ON REPRODUCTION IN PLANTS:

Sulphur dioxide impairs reproduction by damaging pollen, seeds and seedlings. It can also reduce the viability of seeds. These processes were reviewed by Katz (1939).

Pine pollen is prevented from further development when concentration of sulphur-dioxide rises to 200 ppm. Scheffer and Hedgecock (1955) reported the near absence of cone production by damaged pine, and the lack of seedlings and saplings in damaged stands near Trial British Columbia smelter. Pollen tube elongation was inhibited as reported by Masaru et al., (1976). Pollen germination was adversely affected by sulphur-dioxide pollution in Cicer arietenum and Petunia alba, as reported by Varshney and Varshney (1981). Germination of red pine was decreased by exposure of seeds to 100 ppm sulphur-dioxide (Riding and Boyer, 1983).

EFFECT OF SULPHUR-DIOXIDE ON YIELD:

Thomas (1951) indicated a significant loss in yield in plants affected by sulphur-dioxide. Brisley and Jones (1950) fumigated wheat crop by sulphur-dioxide and found that 2-3 fumigations produced 60% leaf area destruction and thereby reduced the yield to 82% of the control. Yield of rye grass showed a decrease when the plants were grown in chambers containing sulphur-dioxide at concentrations insufficient to cause visible injury (Bell and Clough, 1973) and Bleasdale (1973). In 1975, a considerable decrease in yield of rice due to sulphur-dioxide by Taniyama and Sawanaka in Yokkaichi City. 10-12% yield losses have been reported in the agricultural lands near Ohio by Page and Arobogast (1982).

OXIDES OF NITROGEN

Any combustion process, which produces high temperature in the presence of nitrogen and oxygen yields oxides of nitrogen. These oxides include Nitric oxide (NO), nitrogen dioxide (NO_2) and nitrogen tetroxide (N_2O_4).

SOURCES OF NITROGEN OXIDES:

Over 70% of NO_2 in the atmosphere originates from automobile exhausts (Treshow, 1970). Significant amounts are contributed by combustion of fuels for industrial and domestic purposes. Concentration of oxides varies with temperature. Sukuck et al., (1966) have shown the average monthly concentration of oxides of nitrogen in Los Angeles to be 20-30 pphm during the winter and about 10 pphm in the summer.

The biological sources producing oxides of nitrogen are bacteria and man. According to Robinson and Robbins (1970), NO and NO_2 produced by bacteria amount to 50 x 10^7 tonnes/yr and the amount produced by man is about 5 x 10^7 tonnes/year.

MECHANISM OF INJURY:

Palisade cells of the leaf are the most readily injured.

When NO_2 reaches the spongy parenchyma cells, it reacts with water, it forms a mixture of nitrous and nitric acids. As the level of these acids increases beyond a given threshold value, the tissues are injured.

PLANT RESPONSES:

NO_2 injury in the field has been reported in Italy (Janone, 1954) to cause necrotic stem lesions, defoliation, dieback and death of peach and black locust trees. Maclean et al. (1967) described some of the drastic effects of exceedingly high NO_2 concentration. At 10-250 ppm for a duration of 10 min. to 8 hrs, NO_2 caused rapid tissue collapse, necrosis, and total defoliation.

Benedict and Breen (1955) reported two types of injury caused by oxides of nitrogen. Firstly, small irregularly shaped, white or brown necrotic lesions appear between the large secondary, a waxy, glossy sheen develops on both the adaxial and abaxial leaf surfaces.

FLUORIDES AND THEIR EFFECTS ON PLANTS

SOURCES:

Fluoride is abundantly present in the earth's crust as a natural component of soil and rocks, and minerals and when these materials are heated, toxic quantities of fluorides are released into the atmosphere.

ENTRY OF FLUORIDES INTO THE PLANTS:

Stomata provide the main path for the entry of fluorides into the leaf from the air. On coming in contact with the mesophyll cells of the leaf, the fluorides are either directly absorbed by the cells or get transported through the vascular tissues to the tips and leaf margins. Zimmerman and Hitchcock (1956) found that the leaf tips of gladiolus accumulate as much as 25-100 times as much fluoride as the basal section. Chloroplasts are the major site of fluoride accumulation (Chang and Thompson, 1966), though appreciable amounts get accumulated in the cell wall, nuclei and cytoplasm also (Ledbetter et al., 1960).

Some plants, for example those belonging to the camellia family, of which tea is a member, accumulate large amounts of fluorides from the soil. Soluble forms of fluorides, such as sodium fluoride (NaF), potassium fluoride (KF) and hydrogen fluoride (HF) are absorbed and stored in roots and leaves.

EFFECTS OF FLUORIDES ON PLANTS:

Plants develop certain characteristic symptoms of injury due to presence of high concentration of fluorides in the atmosphere. Broad leaved species show necrosis, chlorosis or both, at the leaf tips or margins. The injury first appears as dull grey-green and wet discolouration of the affected tissue which later turns light to dark brown, as the concentration of fluorides reach high toxic levels.

In monocots, fluoride injury may produce reddish-brown waves on the leaves, or the zonation may be absent and necrosis may be seen to appear first at the tips and extend down on one side of the leaf more than the other side. Corn and sorghum show symptoms such as chlorotic stipple or mottle.

Solberg et al. (1955) carried out anatomical studies on ponderosa pine needles affected by fluorides in the atmosphere and found that tissue collapsed only a few cells in advance of the necrotic areas. Further, they found that epidermis, hypodermis and xylem tissues were most resistant and parenchyma most sensitive. Phloem cells were injured first. Phloem and xylem parenchyma become greatly enlarged and later get distorted. The protoplast then became granulated, vacuolated and finally collapsed. In Resin ducts, epithelial cells get enlarged and finally block the ducts.

Flowers are very resistant to fluorides. However, fruits are more sensitive. Soft suture disease is caused in peaches is developed due to fluoride injury, when the fruits show premature reddening and ripening along the sutures of the fruit. Bolay and Bovay (1965) described appearance of a local necrosis, at the stylar end of fruits such as apricots, cherries and pears, due to fluoride injury.

EFFECT ON PLANT GROWTH AND PRODUCTIVITY:

The effects of fluorides on plants need not always be adverse. Aso (1906) concluded that NaF stimulated vegetative growth of barley and pea plants.

Adams and Sulzback (1961) reported that bean plants when fumigated with fluoride exhibited a longer initial internodal growth than the control plants.

Regarding the adverse effects of fluorides on plants, Anderson (1966), showed that leaf size of woody plants such as asper and grape were reduced as low as 30%. Similar results were obtained by Brewer (1960) on orange trees.

Since fluorides cause reduction in leaf size, thereby reducing the photosynthetic area, reduction in yield naturally follows. Merrill Pack in 1966 carried out experiments on tomato plants in Washington State University and showed that HF caused significant reductions in the average weight of fruit per plant and also in the average weight per fruit.

OZONE

Ozone is present in the upper layers of the atmosphere where it plays the role of absorbing and filtering out dangerous ultraviolet radiation. However, it is also produced as a consequence of combustion processes. Light energy splits the nitrogen-dioxide molecule into nitric oxide and nascent oxygen; nascent oxygen combined with oxygen in presence of any inert molecule to form ozone (O_3)

$$NO_2 \xrightarrow{h\nu} NO + O; \quad O + O_2 \xrightarrow{m} O_3$$

where m is any inert molecule and $h\nu$ is light energy.

The net reaction is:

$$NO_2 + O_2 \xrightleftharpoons{h\nu} NO + O_3$$

However, ozone does not persist in the atmosphere because it readily reacts with other chemicals in the atmosphere and is soon neutralized. Depending upon air movements and atmospheric conditions, i.e., temperature and humidity, ozone can travel a long distance and injureplants (Heggestad and Heck, 1971).

ENTRY OF OZONE INTO THE PLANT BODY:

On coming in contact with ozone, the stomatal apertures close, but this process is not fast enough to prevent ozone entry into the leaf. One having achieved entry into the leaf, ozone disrupts the permeability of the cell walls and next it attacks cellular enzymes and organells; thus interfering with the physiological processes.

EFFECT ON PLANTS:

Ozone impairs photosynthesis, respiration and other metabolic processes. This reflects on the overall plant growth. Besides chlorosis, necrosis and mottling of the leaves, it was observed by Hill et al., in 1961 that ozone caused hypertrophy in certain plants. Premature senescence and fruit damage are other outcomes of ozone injury.

ANATOMICAL RESPONSES TO AIR POLLUTION

Air pollution has been reported to effect every part of the plant and thereby it interferes with the overall physiology of the organism. As reported by Khan et al. (1984 a) some outstanding anatomical variations due to air pollution have been brought to light in *Melilotus indica*. There is a considerable increase in the cortex and pith regions, pore number and pore area of the plant, as compared to the control plants. However, the length of vessel and fibres suffers a setback. The same authors have shown that *Polygonum glabrum* and *Chenopodium album* exhibit reduced cortex, pith, secondary xylem and pore area in polluted atmosphere. The number of pores, length and width of vessel segments and fibres also have been shown to decrease over that of the control plants. Ahmad, et al. (1984) reported that wood quality and micromorphology of wood components of *Mangifera indica* were highly influenced by air pollutants. There was a reduction in pore number and pore area. Further, sapwood fibres showed a great decrease in length as compared to heartwood fibres. An annual loss in wood formation amounting to 26% in its average over a period of six years was observed in *Tectona grandis* by Ghouse et al (1984).

Epidermal system has also been found to be influenced by air pollutants. The size and density of trichomes/unit of the leaf area showed an increase in *Croton bonplandianus* Baill., while the stomatal frequency showed a decrease with the increase of pollution (Zaidi et al., 1979). However, the stomatal size and trichome type did not vary. Similar results were obtained by Ghouse et al (1980) in *Callistemon citrinus*.

PLAN OF WORK

Work regarding the problem 'Anatomical responses of a grassland vegetation to Air Pollution' will be carried out in the following sequence, using the parameters listed below:

1) Shoot height
2) Root length
3) Plant height
4) Number of internodes
5) Internodal length
6) Shoot circumference
7) Average leaf area
8) Average fresh weight of leaves/plant
9) Area estimation of the various tissues produced per plant.
10) Amount of secondary xylem
11) Vessel number/unit area of xylem
12) Length and width of vessel segments.
13) Fibre length and amount.
14) Xylem parenchyma
    a) Axial parenchyma
    b) Amount and distribution
15) Ray development and frequency

16) Ground tissue, development and its extent.

17) Cortical sclerenchyma

18) Collenchyma

19) Epidermal features

SELECTION OF SITES:

For the collection of the plants, sites/field stations of almost similar ecological field conditions, particularly the edaphic ones will be chosen. The first station will be chosen within a range of half a km range from the source of pollution. The second station which will serve as control site will be selected within the University campus in pollution free atmosphere. The former site will be named as 'A' and the latter as 'B'.

SOURCE OF POLLUTION:

The work shall be carried out at Kasimpur where a Thermal Power Plant Complex is situated. The location of site A will be near the Thermal power plant complex, whereas site B will be in Aligarh, which is at a distance of 16 km. from Kasimpur.

COLLECTION OF PLANTS:

For growth parameters, 10 plants for each species will be collected from each site, whereas five replicates will be collected for anatomical investigation. Specimens will be collected at different stages of growth, viz: seedling stage, stage of vegetative growth, flowering and fruiting stage.

FIXATION AND PRESERVATION:

The stem pieces from third internode, as well as apex (first internode) will be collected, and preserved in FAA. The samples will be allowed to remain in the fixative for about a week and then transferred to 70% ethanol for preservation.

SECTIONING:

Transverse sections of the fixed samples of the materials will be made on a Reichard's sliding microtome at a thickness of 10-12 µm. Delicate portions such as the shoot apices and leaves shall undergo paraffin wax embedding before sectioning on a Rotary microtome at a thickness of 8-12 µm.

STAINING:

The following staining combinations will be used for staining different tissue systems.

(1) For xylem:

(a) Heidenhain's Iron Haematoxylin and Bismark Brown (Johan-son, 1948).

(b) Iron Haematoxylin and Safranin.

(2) <u>For phloem</u>:

Tannic-acid-ferric chloride-lacmoid (Cheadle et al., 1953).

(3) <u>For leaves</u>:

Safranin and fast green.

MACERATION:

(a) <u>Maceration of xylem</u>:

The stem pieces shall be cut into thin tangential slices of 0.5 to 1 mm. thickness, treated with 20% nitric acid and boiled till the elements get separated. Then the macerated material will be washed thoroughly in distilled water and mounted in 1% glycerine after staining with safranin (Ghouse and Yunus, 1972).

(b) <u>Maceration of phloem</u>:

The thin slices shall be treated with 5% sodium hydroxide solution for 3-5 days at 45-50°C to soften the tissues. Then they shall be transferred to fresh NaOH solution of the same concentration. The slices shall be checked from time to time to see the softness of the tissues. Once the maceration reaches the desired stage, the slices shall be washed and stained in 1% aqueous solution of astra blue for 12-24 hours for sieve-tube elements, mounted in 50% glycerine and then mounted in Canada Balsam.

For the study of fibres, the macerated elements shall be stained in safranin, put in 50% glycerine and mounted in Canada Balsam.

FOLIAR ANATOMICAL STUDIES:

To study the leaf anatomy, serial sections shall be cut after wax embedding (Sass, 1958). For preparation of slides of epidermal peels, the methods given by Ghouse and Yunus (1972) and Ram and Nayyar (1974) shall be adopted.

STATISTICAL ANALYSIS

To an ecologist, statistical technique is an important tool to know the validity and nonvalidity of his data. The data on weed species collected on different aspects of investigation carried out at all the field stations will be statistically analysed as follows:

MEAN ($\overline{X}$)

The arithmetic mean, or simple mean or the so called average value, is easily computed by taking the sum of a number of values (X_1, X_2, X_3 ... and so on) (Σ), and dividing by the total number of values (N) involved; thus,

$$\overline{X} = \frac{(X_1 + X_2 + X_3 \ldots\ldots X_n)}{N} \quad \text{or,} \quad \overline{X} = \frac{x}{N}$$

where, $X_1, X_2, X_3, \ldots\ldots X_n$ = observations

N = Number of observations.

STANDARD DEVIATION (S.D.)

Standard deviation will be utilized to determine statistical significance and calculate correlation coefficient. The standard deviation (or S.D.) will be calculated by the following formula for each parameter of study.

S.D. for large samples :

$$S.D. = \pm\sqrt{\frac{(\overline{X}-x_1)^2 + (\overline{X}-x_2)^2 + (\overline{X}-x_3)^2 + \ldots\ (\overline{X}-x_n)^2}{N}}$$

S.D. for small samples :

$$S.D. = \pm\sqrt{\frac{(\overline{X}-x_1)^2 + (\overline{X}-x_2)^2 + (\overline{X}-x_3)^2 + \ldots\ldots\ (\overline{X}-x_n)^2}{(N-1)}}$$

where, $\overline{X}$ = Mean of the observations involved.

$x_1, x_2, x_3 \ldots x_n$ = Observations.

N = Number of observations.

STANDARD ERROR (S.E. or $\bar{X}$) of Mean

Standard error will be computed by using the value of standard error and be expressed in $\pm$ as that of standard deviation. The following formula will be used:

$$S.E. = \frac{S.D.\ \text{of samples}}{\sqrt{N - 1}}$$

Standard error of mean is, in fact, a measure of reliability of a sample mean as an estimate of the population mean. Standard error of a mean is nothing but the standard deviation of the means, which is a measure of fluctuations in sample means produced as a result of chance factors of sampling from the same population.

STANDARD ERROR OF DIFFERENCE OF SAMPLE MEANS (S.E.D.)

It may be defined as the standard deviation computed from the differences between a large number of pairs of means of randomly selected samples from two populations. Standard error of the differences of two samples viz. $\bar{X}$ and $\bar{Y}$ of two different populations is of great importance when it is to be judged whether or not they differ significantly. It will be computed as follows:

$$S.E.D. = \sqrt{\frac{(S.D._1)^2}{n_1} + \frac{(S.D._2)^2}{n_2}}$$

where, $S.D._1$ = Standard deviation of one sample.

$S.D._2$ = Standard deviation of the other sample.

n_1 = Number of observations of one sample.

n_2 = Number of observations of other sample.

COEFFICIENT OF VARIATION (C.V.)

Coefficient of variation measures the relative magnitude of variation present in observations relative to the magnitude of their arithmetic mean. It is defined as the ratio of standard deviation to arithmetic mean expressed as a percentage, and is a unit less number. The following formula will be applied to compute the C.V.

$$C.V. = \frac{\text{Standard deviation}}{\text{Arithmetic mean}} \times 100$$

or

$$C.V. = \frac{S.D.}{\bar{X}} \times 100$$

where, S.D. = standard deviation of either sample or of population, of whose C.V. is to be taken.

$\bar{X}$ = Arithmetic mean.

TESTS OF SIGNIFICANCE:

Test of significance is a device to find out whether or not an observed pair of means differs significantly from each other, or this difference is a result of chance influence. A test of significance is a device - a criterion - to arrive at a judgement with confidence about the validity of a result. The following two tests will be applied to have confidence in the validity of the results.

STUDENTS t-TEST OR T-TEST:

This test is applied to test the difference observed between two sample means. In the present study, it will be applied to test the significance of the difference between the two sample means (if any), each sample collected from the two field stations.

The following formula will be used to compute t-values, which will be compared with the table values of 't' at their particular degrees of freedom. If calculated t-value exceeds the table value, the difference between the two samples will be as significant, otherwise the difference will be attributable to chance factors.

$$t = \frac{\text{Difference of two sample means}}{\text{Standard error of the difference}}$$

or, $$t = \frac{\bar{X}_1 - \bar{X}_2}{\sqrt{\frac{(SD_1)^2}{n_1} + \frac{(SD_2)^2}{n_2}}}$$

where, $\bar{X}_1$ = Arithmetic mean of one sample.

$\bar{X}_2$ = Arithmetic mean of the other sample.

SD_1 = Standard deviation of one sample.

SD_2 = Standard deviation of other sample.

n_1 = Number of observations of one sample

n_2 = Number of observations of the other sample.

DEGREES OF FREEDOM (D.F.):

Degrees of freedom, to be applied to number of data, particularly in t-test will be calculated as follows:

$$D.F. = n_1 + n_2 - 2$$

where, n_1 = Number of observations of one sample.

n_2 = Number of observations of the other sample.

Above is applied in t-test, whereas the following in Least Significant Difference analysis (L.S.D.)

$$D.F. = [(T \times R) - 1] - [(R - 1) + (T - 1)]$$

where, T = Number of treatments.

R = Number of observations.

LEAST SIGNIFICANT DIFFERENCE (L.S.D.):

This test is applied to compare all pairs of means. The following formula will be used to calculate L.S.D.:

$$L.S.D. = \sqrt{\frac{2 \times MSE}{r}} \times \text{t-value}$$

where, MSE = Estimated variance of error.

r = Number of replicates.

$$MSE = \frac{SSQE}{(r - 1)(t - 1)}$$

where, SSQE = Error sum of squares.

r = Number of replicates

t = Number of treatments.

$$SSQE = SSQT - (SSQr - SSQt)$$

where, SSQT = Total sum of squares.

SSQr = Sum of square between replications.

SSQt = Sum of square between treatments.

SSQT = Sum of the squares of each value and substrated from it correction factor (C.F.).

where, $$C.F. = \frac{(\text{Total})^2}{r \times t}$$

$$SSQr = \frac{\text{Sum of squares of replications}}{(\text{Number of treatments} - 1)} - CF$$

$$SSQt = \frac{\text{Sum of squares of treatments}}{(\text{Number of replications} - 1)} - CF$$

CORRELATION COEFFICIENT:

A correlation coefficient is a statistical measure which indicates both nature and degree of relationship between two measurable characteristics, say height (X - cm) and yield (Y - gm). It is generally denoted by symbol r. It will be computed as follows:

$$r = \frac{N \sum XY - (\sum X)(\sum Y)}{\sqrt{\left[N \sum X^2 - (\sum X)^2\right]\left[N \sum Y^2 - (\sum y)^2\right]}}$$

where, X = Observations of height.

Y = Observation of yield.

There is another alternative formula for determining the coefficient of correlation as follows:

$$r = \frac{(X - \bar{X})\ (Y - \bar{Y})}{\sqrt{(X-\bar{X})^2 (Y-\bar{Y})^2}}$$

where, X = Observations of one character.

$\bar{X}$ = Arithmetic mean of all X observations.

Y = Observations of other character.

$\bar{Y}$ = Arithmetic mean of all Y observations.

A correlation coefficient may vary from - 1 (perfect negative correlation) to +1 (perfect positive correlation). Any value close to zero would denote a lack of correlation or a relatively low correlation.

COEFFICIENT OF DETERMINATION:

It is a derivative of correlation coefficient and denoted by (d). When expressed in percentage, it shows percent of variation in other variable. The complementary value for this is called coefficient of non-determination. This shows the extent to which a character e.g. height is not responsible for variations in other character e.g., yield. Both will be determined as follows:

$$d = (\ r\ )^2$$

or $$d = 100\ (r)^2$$ - expressed in percentage.

where, d = Coefficient of determination.

r = Correlation coefficient.

$$dn = (1 - r^2)$$

or $$dn = 100\,(1-r^2)$$ - expressed in percentage

where, dn = Coefficient of non-determination.

r = Correlation coefficient.

LINEAR REGRESSION:

Correlation coefficient elucidates the nature and degree of relationship between two characteristics. Due to such correlations when variations in one variable bring in accompanying changes in other, it enables us to predict the value of one variable from the knowledge of other.

The regression line 'best' fitting the observations is given by:

$$\hat{Y} = a + bX$$

$$b = \frac{N \; EX - (\;X)(\;Y)}{N \; X^2 - (\;X)^2}$$

$$a = \bar{Y} - b\bar{X}$$

where, $\hat{Y}$ (Y-hat) indicates the predicted value of Y for a given value of X.

X, Y are observations of two variables, viz., height and yield a, b are the constants.

$\bar{X}$, $\bar{Y}$ are arithmetic means of all observation of respective variables X and Y.

COMPUTER PROGRAMME:

A computer programme will be prepared, based on the above formula and the data will be analysed by the computer.

GEOGRAPHY AND METEROLOGY:

The geographical condition of the study site will be explained and meterological data will be collected from various reliable sources. The results will be interpreted with reference to local climatic and geographical conditions.

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